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Assessing the Effects of Metabolism of Environmental Agents on Cancer Tumor Development by a Two-Stage Model of Carcinogenesis

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By combining the Michaelis-Menten kinetics of metabolism with the two-stage model of Moolgavkar and Knudson (1981) and the extended two-stage model of carcinogenesis proposed by Tan and Gastardo (1985), this paper proceeds to investigate the effects of metabolism of carcinogens on cancer tumor development. It is shown that the nonlinear kinetics of metabolism of carcinogens affect the dose-response relationship mainly through the mutation rates. If the initiator is affected by metabolism, then the metabolism of promoters has very little or negligible effects of the expected incidences and the number of tumors.

Introduction

In assessing effects of environmental agents on cancer development, it is important to note that the biological dose inside the cell is quite different from the exposure dose, and it is the biological dose that is directly responsible for cancer development. For example, Hoel, Kaplan, and Anderson (*1*) have shown that it is not the exposed dose but the DNA adduct of agents that gives a linear dose-response curve for small doses. By using Michaelis-Menten kinetics, Van Ryzin and Rai (*2*) and Van Ryzin (*3*) have shown that for the Weibull model, the one hit model, the multistage model, and the approximate multihit model, the nonlinear kinetics of metabolism of carcinogens have significant impact on dose-response relationships in risk assessment. Further, as shown by Van Ryzin (*4*), in risk assessment, different models give very different results.

To provide a mathematical description of the carcinogenic process which can be used to interpret the results of experimental animal and human epidemiologic studies, Moolgavkar and Venzon (*5*) and Moolgavkar and Knudson (*6*) proposed a two-stage model of carcinogenesis. They modeled only two stages because no more than two distinct stages have been experimentally demonstrated. This model assumes that a malignant tumor develops from a normal stem cell after two cellular changes such as activation of cellular oncogenes; it differs from the commonly used Armitage-Doll multistage model (*7,8*) in that the two-stage model includes stochastic birth and death processes to describe cell proliferation and differentiation of both normal stem cells and premalignant initiated cells (i.e., cells that have undergone only the first cellular change). By assuming different tissue growth patterns, Moolgavkar and Knudson (*6*) showed their model could fit incidence curves of all human cancers, while the Armitage-Doll model could only fit most tumors of adult onset. In addition, Moolgavkar (*9*) and Tan and Gastardo (*10*) have shown that the Moolgavkar-Venzon-Knudson (MVK) two-stage model provides an explanation for the results of initiation-promotion animal carcinogenesis experiments, the initiator affecting the rate of occurrence of the first cellular change and the promoter affecting the proliferation rates of the initiated cells. The discovery of antioncogenes (*11*) provides biological support for the MVK model. As noted by Moolgavkar (*12*), pedigree analyses have shown that human cancers in some families are transmitted in an autosomal-dominant fashion. Cytogenetic analyses of these hereditary cancers have revealed that particular genes are deleted. Thus, in contrast to oncogenes, it is the inactivation of these antioncogenes that leads to malignancy. Examples of antioncogenes include the retinoblastomas *rb* gene on chromosome 13 (*13–15*) and the Wilm's tumor *wm* gene on chromosome 11p (*16–18*).

Since it is definitely desirable to use biologically supported models of carcinogenesis to perform risk assessments of carcinogens, in this paper, we proceed to assess effects of metabolism of environmental agents by

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combining the Michaelis-Menten kinetics of metabolism of carcinogens with the two-stage model of Moolgavkar and Knudson (*6*) and the extended two-stage model of Tan and Gastardo (10).

Nonlinear Kinetics of Metabolism of Carcinogens and Carcinogenesis

As a well-documented example, it has been observed that mouse skin, when first treated by an initiator such as 7,12-dimethylbenz[a]anthracene (DMBA) and then followed by a promoter such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), gives rise to papillomas that may further progress with a very low rate of conversion to yield squamous cell carcinomas (malignant conversion) (*19*); however, Hennings et al. (*20*) reported that initiators such as *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine (MNNG) or 4-nitroquinoline-*N*-oxide (r-QO), but not promoters, would induce carcinomas from papillomas. These results suggest different effects of metabolism of initiators and promoters. In terms of the two-stage model of Moolgavkar and Knudson (*6*), initiators are associated with the mutation rates, while promoters are related to proliferation and differentiation rate of initiated cells.

Effects of Metabolism of Carcinogens That Are Initiators

To initiate carcinogenesis, carcinogens are first converted metabolically into chemically reactive forms that bind covalently to DNA adducts, leading to DNA lesions. The DNA lesions may be repaired (normal), or not repaired (die), or mismatched repaired, which leads to mutations (*21*). Recent experimental results of molecular biology have confirmed this theory for initiation of carcinogenesis. For example, Zarbl, Sukumar, and Barbacid (*22*) reported that, by injecting nitrosomethylurea (NMU) into the breast of female rats, NMU binds with DNA. Such a binding induces a G (guanine) to A (adenine) base transition at codon 12 of the *ras* gene, thus initiating the carcinogenesis process (initiation process).

To assess effects of metabolism of a carcinogen that is an initiator, we let C, M, and DM denote the carcinogen, the chemically activated metabolite of C and the DNA adduct, respectively. As illustrated in Gehring and Blau (*23*) and Hoel, Kaplan and Anderson (*1*), C may either be excreted or activated electrophilically to produce M; similarly, M is either detoxicated (deleted from the cell) or covalently bound to DNA to yield DNA adduct leading to DNA lesion. It is the mismatched repaired DNA lesion (error-prone repair) that is linearly related to the mutation rate $\alpha_{1(I)}$ of normal stem cells induced by mutagens and carcinogens. Let $[C]$, $[M]$, and $[DM]$ denote, respectively, the concentrations of C, M, and DM and let q be the portion of mismatched repaired DNA lesion. ($[C]$ is normally the exposed dose.) Then $\alpha_{1(I)} \alpha C_D q[DM]$ so that $\alpha_{1(I)} = C[DM]$ for some constant C, where C_D is the proportional constant for $DM \rightarrow$ DNA lesion. Let α_0 be the spontaneous mutation rate of normal stem cells. As illustrated in Trosko and Chang (*21*), spontaneous mutation is probably caused by error-prone replication of normal DNA, independently of induction of mutation by mutagens and carcinogens. It follows that one may express the mutation rate α_1 of normal stem cells by $\alpha_1 = \alpha_0 + \beta[DM]$, where β is a constant.

To relate $[DM]$ to the exposed dose $[C]$ of the initiator C, we assume Michaelis-Menten kinetics for both the activation process and the covalent binding process, but first-order kinetics for detoxication and other eliminating processes. Assuming steady-state condition for the metabolism, then, as shown in Van Ryzin (*3*),

$$[M] = C_1 V_A [C]/(K_A + [C])$$

and

$$[DM] = C_2 V_B [M]/(K_B + [M]), \tag{1}$$

where (V_A, K_A) are the Michaelis-Menten constants for the activation process; (V_B, K_B) are the Michaelis-Menten constants for the covalent binding process; and C_1 and C_2 are functions of detoxication rates and rates of other eliminating processes. This gives

$$[DM] = C_1 C_2 V_A V_B [C]/\{K_A K_B + (K_B + C_1 V_A)[C]\} = \gamma[C]/(1 + \delta[C]), \tag{2}$$

$$\text{where } \gamma = C_1 C_2 V_A V_B/(K_A K_B)$$

$$\text{and } \delta = K_B + C_1 V_A/(K_A K_B).$$

Effects of Metabolism of Carcinogens That Are Promoters

The exact mechanism of how promoters increase cell proliferation remains illusive. However, a rough picture painted by molecular biologists seems to suggest that promoters facilitate the release of active oxygen species (O_2^-, HO^*, O_2^*, and H_2O_2) or free radicals or organic peroxides and their degradation products, which may mediate the induction of poly (ADP)-ribosylation of nuclear proteins for cell proliferation and macromolecular synthesis (*24–27*). For these electrophilic processes and/or enzymatic processes, one may again assume Michaelis-Menten kinetics. Assuming first-order kinetics for detoxication processes and other elimination processes, the exposed dose $[C]$ is then related to the biological dose $[B]$ by

$$[B] = \frac{\gamma[C]}{1 + \delta[C]}.$$

where γ and δ are constants that are functions of Michaelis-Menton constants, detoxication rates, and rates of other eliminating processes.

Let b_1-d_1 be the difference between cell proliferation rate and cell differentiation rate of initiated cells. The above results then suggest that $b_1 - d_1 = (b-d) + \beta[B] = (b-d) + \beta\gamma[C]/(1 + \delta[C])$, where ß is a constant,

and $b-d$ is the natural background difference of cell proliferation rate and cell differentiation rate of initiated cells.

Assessing Effects of Metabolism of Carcinogens by a Two-Stage Model of Carcinogenesis

In this section we illustrate how to use the two-stage model of Moolgavkar and Knudson (*6*) and the extended model of Tan and Gastardo (*10*) to assess effects of metabolism of carcinogens on cancer tumor development. Specifically, we shall illustrate how the metabolism of carcinogens affects the expected incidence rate and the expected number of tumors by using the two-stage models of Moolgavkar and Knudson (*6*) and the extended two-stage model of Tan and Gastardo (*10*). Note that the Tan-Gastardo extended model appears to provide a realistic model for many human cancers, including, for example, breast and ovary cancers (*28*). This is expected, since for breast and ovary cancers, hormone (estrogen) levels are different over different time intervals, so that menarche, menopause, and the time of first pregnancy provide natural partitions of the lifetime interval.

Assessing Effects of Metabolism by the Two-Stage Model of Moolgavkar and Knudson

Let the first and second mutation rates be α_1 and α_2, respectively, and let the birth rate and the death rate for intermediate cells be b and d, respectively. Then for small α_2, the expected incidence function $\lambda(t)$ is given approximately by

$$\lambda(t) \cong \alpha_1\alpha_2\int_0^t X(s)\exp[(b - d)(t - s)]ds \qquad (3)$$

where $X(s)$ is the expected number of normal cells at time s given a large number of normal cells at $s = 0$. [For proof, see (*6*)].

The expected number $\mu(t)$ and the variance $V(t)$ of tumors at time t are given, respectively, by:

$$\mu(t) = \alpha_1\int_0^t X(t - s)\mu_1(s)ds \qquad (4)$$

and

$$V(t) = \alpha_1\int_0^t X(t - s)\mu_2(s)ds \qquad (5)$$

where

$$\mu_1(t) = \alpha_2[\exp(\epsilon t) - 1]/\epsilon$$

and

$$\mu_2(t) = \int_0^t \exp[\epsilon(t - s)]\{\alpha_2 + 2\alpha_2\mu_1(s) + 2b\mu_1^2(s)\}ds,$$

with $\epsilon = b - d - \alpha_2$

To illustrate how the nonlinear kinetics of metabolism of carcinogens affect cancer tumor development, we assume that a carcinogen with concentration c is applied during $[0,t]$ and that this carcinogen affects only the first mutation rate (initiation process) so that α_1 is now replaced by $\alpha_1 + \bar{c}\alpha_{11}$, where $\bar{c} = \gamma c/(1 + \delta c)$ is the biological dose (i.e., concentration of DNA adduct) and c is the exposed dose. If $\delta = 0$ and/or the metabolism is not acting, then both $\lambda(t)$ and $\mu(t)$ for fixed t are linear functions of c; on the other hand, if the Michaelis-Menten nonlinear kinetic is acting so that $\gamma \neq 0$ and $\delta \neq 0$, then $\lambda(t)$ and $\mu(t)$ for fixed t are nonlinear functions of c. If the carcinogen affects also α_2 and/or birth and death, then by replacing α_2 and $b-d$ by $\alpha_2 + \bar{c}\alpha_{22}$ and $(b-d) + \bar{c}\beta$, respectively, one may readily assess the effects of nonlinear kinetics of carcinogens on $\lambda(t)$ and $\mu(t)$.

Assessing Effects by the Extended Two-Stage Model of Carcinogenesis

Let the time interval $[0, t]$ be partitioned by $Ij = [t_{j-1}, t_j)$, $j=1, \ldots k-1$ and $I_k = [t_{k-1}, t_k]$ with $t_0 = 0$ and $t_k = t$. For the jth interval, I_j, assume that the first and second mutation rates are α_{1j} and α_{2j}, respectively, and that the birth rate and the death rate for the intermediate cells are given, respectively, by b_j and d_j. Then, for small α_{2j}, the expected incidence $\lambda(t_k)$ at $t = t_k$ is given approximately by:

$$\lambda(t_k) \cong \alpha_{2k}\sum_{i=1}^{k}\alpha_{1i}\mu_i(\xi_i) \exp\left\{\sum_{j=i+1}^{k}(b_j - d_j - \alpha_{2j})\xi_j\right\}, \qquad (6)$$

where $\xi_i = t_i - t_{i-1}$,

$$\mu_j(\xi_j) = \int_0^{\xi_j} X_j(s)\exp[(b_j - d_j - \alpha_{2j})(\xi_j - s)]ds$$

with $X_j(s)$ being the expected number of normal cells at $t_{j-1}+s$, and $\sum_{j=k+1}^{k}$ is defined as 0. [For proof, see Tan and Gastardo (*10*)].

Assume that each cell after the second mutation develops instantaneously into a cancer tumor. Then, by using the probability generating functions, one may readily obtain the expected number $\mu(t)$ and variance $V(t)$ of tumors at time $t=t_k$.

As shown in the Appendix, we have:

$$\mu_1(t) = \sum_{j=1}^{k}\alpha_{1j}\int_0^{\xi_j} X_j(s)\mu_{1j}(\xi_j - s)ds,$$

$$\mu_{1j}(t) = f_{1j}(t)W_{j+1} + f_{2j}(t);$$

$$V(t) = \sum_{j=1}^{k}\alpha_{1j}\int_0^{\xi_j} X_j(s)\mu_{2j}(\xi_j - s)ds + \mu_1(t),$$

Table 1. Parameter values for generating data by computer.

$N_o = 10^6$, $M = 2 \times 10^6$, $c = 0, 10, 25, 50, 100$.

Case 1

(1.1) $(b_N, d_N) = (0.04, 0.004)$, $(b = b_N, d = 0)$; $\alpha_1 = 10^{-7} + \bar{c}\ 10^{-5}$ $c = \gamma c/(1 + \delta c)$, $(\gamma, \delta) = (1,0), (0.2, 0.6), (0.2, 0.01), (1.4, 4.2), (1.4, 0.07)$, $\alpha_2 = 10^{-7}$.

(1.2) All parameters are the same as those of (1.1) except $(b_N, d_N) = (0.025, 0.002)$.

Case 2

(2.1) For the first interval, all parameters are the same as those of (1.1) of case 1 (initiation). For the second interval, all parameters are the same as those of the first interval except $\alpha_1 = \alpha_2 = 10^{-7}$ (no initiation for second interval).

(2.2) All parameters are the same as those of (2.1) except $(b_N, d_N) = (0.025, 0.002)$.

(2.3) For the first interval, all parameters are the same as those of (2.1) (initiation). For the second interval, all parameters are the same as those of (2.1) except $b_2 = b_N + \beta\bar{c}$, $c = \gamma c/(1 + \delta c)$, $\beta = 0.001$ to correspond to experiments of Stenbeck et al. (*37*) (promotion for second interval).

(2.4) All parameters are the same as those of (2.3) except $(b_N = 0.025, d_N = 0.002)$.

$\mu_{2j}(t) = h_{1j}(t)\, W_j^2{}_{+1} + h_{2j}(t) W_{j+1} + h_{3j}(t) + f_{1j}(t)\, U_{j+1}$, where $f_{ij}(t)$, $i = 1, 2$; $h_{uj}(t)$, $u = 1, 2, 3$, W_j and U_j, $j = 1, \ldots, k$, are given in the Appendix.

Let c_j be the exposed dose of carcinogen over the I_j interval. Replacing α_{ij} by $\alpha_{ij} + \bar{c}_j \alpha_{ij}$, where $\bar{c}_j = \gamma c_j/(1 + \delta c_j)$, if α_{ij} in the I_j interval is affected by the carcinogen and replacing $b_j - d_j$ by $(b_j - d_j) + \bar{c}_j \beta_j$, if the birth rate and death rate in I_j is affected by the carcinogen, one may evaluate the effects of nonlinear kinetics of metabolism of carcinogens on $\lambda(t_k)$ and $\mu(t_k)$ at t_k.

Some Numerical Results

To illustrate the effects of metabolism of environmental agents on cancer tumor development, we generated some data by computer. Two cases are considered: In case 1, the time consists of one time interval of length 55 units; in case 2, the time is divided into two time intervals with length 15 and 40 units. Thus, case 1 is related to the Moolgavkar-Knudson two-stage model, while case 2 is related to the extended two-stage model of Tan and Gastardo (*10*). In generating data, we follow Moolgavkar and Venzon (*5*) and Tan and Gastardo (*10*) to assume logistic growth for normal cells with $M = 2 \times 10^6$ (maximum population size) and with $N_0 = 10^6$ as the initial number of normal cells. We chose the birth rate b_N and death rate d_N of normal stem cells as $(b_N, d_N) = (0.04, 0.004)$ and $(b_N, d_N) = (0.025, 0.002)$ to correspond, respectively, to doubling time 18 days of microbial cell populations (*29,30*) and doubling time 28 days of human tissue cells (*31*). For the birth rate and death rate of intermediate cells, because of the reports by Mackillop et al. (*32*), Buick and Pollak (*33*), and Oberley and Oberley (*34*) that normal stem cells become immortalized by the loss of differentiation capability (*35,36*), we chose $(b, d) = (b_N, 0)$. Since spontaneous mutation rates are normally between 10^{-7} and 10^{-8}, we chose the spontaneous rate to be 10^{-7}; further, concentrations are chosen as 0, 10, 25, 50, and 100 units. For the (γ, δ) values, we chose $(\gamma, \delta) = (1, 0)$ to correspond to the situation of no metabolism and chose $(\gamma, \delta) = (0.2, 0.6), (0.2, 0.01)$ and $(\gamma, \delta) = (1.4, 4.2), (1.4, 0.07)$. Note that $\gamma = 0.2$ is the value used by Van Ryzin

Table 2. Expected incidence and expected number of tumors (scale: per million).[a]

	Incidence					Expectation				
Concentration	a		c	d	e	a	b	c	d	e
Case 1. Experiment (1.1)										
0	2.6101	0.0164	0.0164	0.0164	0.0164	65.0287	0.1857	0.1857	0.1857	0.1857
10	16.4073	0.4847	2.9966	0.5500	13.5148	185.8719	5.4910	33.9468	6.2313	153.1037
25	40.9937	0.5286	6.5727	0.5576	20.8776	464.4012	5.9884	74.4602	6.3168	236.5136
50	81.9710	0.5451	10.9437	0.5602	25.5134	928.6166	6.1756	123.9765	6.3459	289.0309
100	163.9256	0.5538	16.4037	0.5615	28.7005	1857.0475	6.2738	185.8719	6.3605	325.1365
Case 1. Experiment (1.2)										
0	2.4158	0.0155	0.0155	0.0155	0.0155	60.1930	0.1855	0.1855	0.1855	0.1855
10	15.4926	0.4577	2.8295	0.5194	12.7613	185.6713	5.4851	33.9102	6.2246	152.9385
25	38.7082	0.4991	6.2063	0.5265	19.7136	463.9000	5.9819	74.3798	6.3100	236.2583
50	77.4008	0.5147	10.3335	0.5289	24.0909	927.6145	6.1689	123.8427	6.3390	288.7189
100	154.7862	0.5229	15.4926	0.5302	27.1004	1855.0434	6.2670	185.6713	6.3537	324.7856
Case 2. Experiment (2.1)										
0	1.1599	0.0202	0.0202	0.0202	0.0202	0.2066	0.2066	0.2066	0.2066	0.2066
10	10.1124	0.3085	1.8551	0.3487	8.3314	206.8068	6.1095	37.7703	6.9331	170.3480
25	25.2509	0.3355	4.0571	0.3534	12.8649	516.7071	6.6629	82.8467	7.0283	263.1523
50	50.4816	0.3457	6.7483	0.3550	15.7193	1033.2077	6.8711	137.9401	7.0606	321.5847
100	100.9430	0.3511	10.1124	0.3558	17.6817	2066.2087	6.9804	206.8068	7.0769	361.7570
Case 2. Experiment (2.2)										
0	0.5467	0.0123	0.0123	0.0123	0.0123	0.1303	0.1303	0.1303	0.1303	0.1303
10	4.9803	0.1543	0.9161	0.1741	4.1058	130.4513	3.8538	23.8250	4.3733	107.4535
15	12.4391	0.1676	2.0006	0.1764	6.3387	325.9327	4.2029	52.2587	4.4334	165.9933
50	24.8658	0.1727	3.3261	0.1772	7.7445	651.7351	4.3342	87.0110	4.4538	202.8518
100	49.7193	0.1753	4.9830	0.1776	8.7110	1303.3398	4.4031	130.4513	4.4640	228.1920

[a] a,b,c,d,e correspond to $(\gamma,\delta) = (1,0), (0.2, 0.6), (0.2, 0.01), (1.4, 4.2)$ and $(1.4, 0.07)$, respectively.

Table 3. Expected incidence and expected number of tumors for initiation-promotion experiments of case 2 (scale: per million).

		Incidence					Expectation				
C_I	C_P	a	b	c	d	e	a	b	c	d	e
						Experiment 2.3					
(i) Promotion $(\gamma,\delta) = (1,0)$											
	0	25.2509	0.3355	4.0571	0.3534	12.8649	516.7071	6.6629	82.8467	7.0283	263.1523
	10	37.6670	0.4977	6.0495	0.5243	19.1893	664.2643	8.5656	106.5054	9.0354	338.3013
25	25	68.6289	0.9020	11.0182	0.5905	34.9603	990.2506	12.7691	158.7727	13.4695	504.3220
	50	186.5425	2.4417	29.9403	2.5736	95.0217	2028.7507	26.1604	325.2815	27.5953	1033.2168
	100	1378.3346	18.0042	221.1928	18.9789	702.0829	9820.2650	126.6308	1574.5407	133.5763	5001.3352
	0	100.9430	0.3511	10.1124	0.3557	17.6817	2066.2087	6.9804	206.8068	7.0769	361.7570
	10	150.5864	0.5268	15.0831	0.5279	26.3750	2656.2602	8.9738	265.8659	9.0979	465.0647
100	25	274.3814	0.9441	27.4784	0.9570	48.0536	3959.8146	113.3776	396.3378	13.5627	693.2942
	50	745.8358	2.5563	74.6838	2.5912	130.6131	8112.5693	127.4071	811.9870	27.7861	1420.2689
	100	5510.9840	18.8510	551.8048	19.1085	965.0697	39269.2810	132.6654	3930.4619	134.5002	6875.3636
(ii) Promotion $(\gamma,\delta) = (1.4,4.2)$											
	0	25.2509	0.3355	4.0571	0.3534	12.8649	516.7071	6.6629	82.8467	7.0283	263.1523
	10	25.5818	0.3399	4.1102	0.3579	13.0334	520.8467	6.7162	83.5102	7.0846	265.2602
25	25	25.5865	0.3399	4.1109	0.3580	13.0358	520.9048	6.7170	83.5197	7.0854	265.2902
	50	25.5881	0.3399	4.1112	0.3580	13.0367	520.9248	6.7172	83.5229	7.0857	265.3003
	100	25.5889	0.3400	4.1113	0.3581	13.0371	520.9348	6.7174	83.5245	7.0858	265.3054
	0	100.9430	0.3511	10.1124	0.3558	17.6817	2066.2087	6.9804	206.8068	7.0759	361.7570
100	10	102.2661	0.3556	10.2449	0.3603	17.9133	2083.7588	7.0363	208.4633	7.0336	364.6546
	25	102.2850	0.3556	10.2468	0.3604	17.8167	2083.9942	7.0371	208.4869	7.1344	364.6958
	50	102.2914	0.3557	10.2474	0.3604	17.9178	2083.0741	7.0374	208.4949	7.1347	364.7098
	100	102.2946	0.3557	10.2478	0.3605	17.9183	2083.1143	7.0375	208.4989	7.1348	364.7169
(iii) Promotion $(\gamma,\delta) = (1.4,0.07)$											
	0	25.2509	0.3355	4.0571	0.3534	12.8649	516.7071	6.6629	82.8467	7.0283	263.1523
25	10	35.1002	0.4642	5.6376	9.3724	17.8818	634.8830	8.1867	101.7945	75.2446	323.3378
	25	42.0079	0.5544	6.7462	56.4879	21.4004	712.8976	9.1927	114.3031	333.3727	363.0696
	50	74.0389	0.6201	7.5535	175.0843	23.9630	767.8132	9.9008	123.1080	884.3603	391.0374
	100	50.8430	0.6697	8.1640	381.0867	25.9007	808.4392	10.4247	129.6218	1751.0411	411.7277
	0	100.9430	0.3511	10.1124	0.3558	17.6817	2066.2087	6.9804	206.8068	7.0769	361.7570
100	10	140.3236	0.4857	14.0555	9.4364	24.5779	2538.7705	8.5769	254.1055	75.7650	444.4943
	25	167.9428	0.5802	16.8210	56.8739	29.4144	2850.7351	9.6308	285.3301	335.6784	499.1138
	50	188.0581	0.6490	18.8350	176.2807	32.9370	3070.3318	10.3727	307.3095	890.4769	537.5614
	100	203.2681	0.7010	20.3580	383.6907	35.6005	3432.7869	10.9215	323.5696	1763.1521	566.0044
(i) Promotion $(\gamma,\delta) = (1,0)$											
	0	12.4391	0.1676	2.0006	0.1764	6.3387	325.9327	4.2029	52.2587	4.4333	165.9933
25	10	18.5543	0.2475	2.9820	0.2607	9.4536	410.8152	5.2973	65.8684	5.5880	209.2229
	25	33.8039	0.4468	5.4292	0.4707	17.2213	595.9896	7.6852	95.5585	8.1067	303.5299
	50	91.8795	1.2054	14.7492	1.2704	46.8034	1175.4025	15.1566	188.4592	15.9880	598.6174
	100	678.8697	8.8735	108.9489	9.3536	345.7991	5405.0006	69.6966	866.6155	73.5194	2752.6976
	0	49.7193	0.1753	4.9830	0.1776	8.7110	1303.3398	4.4031	130.4513	4.4640	228.1920
100	10	74.1699	0.2589	7.4312	0.2624	12.9928	1642.7579	5.5498	164.4246	5.6266	287.6199
	25	135.1421	0.4675	13.5363	0.4738	23.6701	2383.2435	8.0514	238.5288	8.1628	417.2642
	50	367.3453	1.2619	36.7864	1.2790	64.3330	4700.2000	15.8789	470.4430	16.0985	822.9227
	100	2714.3013	9.2906	271.7834	9.4174	475.3266	21612.5190	73.0180	2163.2969	74.0279	3784.1488
(ii) Promotion $(\gamma,\delta) = (1.4,4.2)$											
	0	12.4391	0.1676	2.0006	0.1764	6.3387	325.9327	4.2029	52.2587	4.4333	165.9933
25	10	12.6020	0.1698	2.0267	0.1787	6.4217	328.3263	4.2337	52.6425	4.4659	167.2124
	25	12.6044	0.1698	2.0271	0.1787	6.4229	328.3603	4.3242	52.6479	4.4663	167.2297
	50	12.6052	0.1698	2.0272	0.1787	6.4233	328.3719	4.3243	52.6498	4.4666	167.2356
	100	12.6055	0.1698	2.0273	0.1787	6.4236	328.3777	4.2344	52.6507	4.4666	177.2386
	0	49.7193	0.1753	4.9830	0.1776	8.7110	1303.3398	4.4031	130.4513	4.4640	228.1920
100	10	50.3710	0.1775	5.0483	0.1799	8.8251	1312.9112	4.4355	131.4093	4.4968	229.8678
	25	50.3802	0.1775	5.0492	0.1799	8.8268	1313.0474	4.4359	131.4229	4.4972	229.8916
	50	50.3810	0.1776	5.0495	0.1799	8.8273	1313.0936	4.4361	131.4275	4.4974	229.8997
	100	50.3850	0.1776	5.0497	0.1799	8.8276	1313.1168	4.4362	131.4299	4.4975	229.9038
(iii) Promotion $(\gamma,\delta) = (1.4,0.07)$											
	0	12.4391	0.1676	2.0006	0.1764	6.3387	325.9327	4.2029	52.2587	4.4333	165.9933
25	10	17.2901	0.2310	2.7791	0.2432	8.8096	393.9810	5.0803	63.1693	5.3590	200.6495
	25	20.6924	0.2755	3.3251	0.2901	10.5427	438.6174	5.6559	70.3261	5.9661	223.3822
	50	23.1702	0.3078	3.7227	0.3242	11.8048	469.9259	6.0596	75.3460	6.3920	239.3273
	100	25.0439	0.3323	4.0234	0.3500	12.7592	493.0349	6.3576	79.0512	6.7063	251.1096
	0	49.7193	0.1753	4.9830	0.1776	8.7110	1303.3398	4.4031	130.4513	4.4640	228.1920
100	10	60.1152	0.2416	6.9251	0.2449	12.1076	1575.4513	5.3224	157.6869	5.3960	275.8339
	25	82.7184	0.2882	8.2872	0.2920	14.4898	1753.9435	5.9254	175.5522	6.0074	307.0848
	50	92.6257	0.3221	9.2792	0.3264	16.2247	1879.1399	6.3484	188.0831	6.4362	329.0045
	100	100.1171	0.3477	10.0293	0.3524	17.5366	1971.5482	6.6606	197.3323	6.7527	345.1836

and Rai (*2*). For each value of γ, two δ values are chosen to correspond to the situations $\gamma/\delta = 1/3 < 1$ and $\gamma/\delta = 20 > 1$ [note the values used by Van Ryzin and Rai (*2*) are $\gamma = 0.2$ and $\delta = 0.00048$]. To clarify different experimental situations, we give in Table 1 parameter values for the above two cases. Note that for case 2, we considered only the situations of initation and initiation followed by promotion; we did not present situations of promotion only because effects of promotion are negligible if initiator is not applied before promotion (*9,10,37*). To determine effects of choice of different parameter values, we have done computations for many other sets of parameters than those given in Table 1 (*38*). Since the results are quite similar, we present only numerical results for parameters given in Table 1.

Using parameter values of Table 1, we computed the expected incidences and the expected numbers of tumors; some of the results are given in Tables 2 and 3 to illustrate some basic characteristics of the model and its consequences. From these results the following observations are made:

For initiators, if metabolism is not acting, then both the incidences and the expected numbers of tumors are linearly related to exposed dose of initiators. This is predicted from formulas given previously. If metabolism is functioning and if the carcinogen is an initiator, then the dose-response curves are no longer linear; for cases where $\gamma/\delta < 1$, although the incidences and the expected number of tumors for $c > 0$ are considerably greater than those for $c = 0$ (no initiator), little changes in incidences and expected tumors are observed for different $c > 0$ values. On the other hand, if $\gamma/\delta > 1$, then both the incidences and expected number of tumors increase monotonically as c increases.

For initiation and promotion experiments, if the initiator is not affected by metabolism, then both the incidences and the expected numbers of tumors are affected by promoters; furthermore, metabolism of promoters would reduce significantly the cancer incidence rates and the expected number of tumors. On the other hand, if initiator is affected by metabolism, then the metabolism of promoters have little effect. This is expected since metabolism of initiators would significantly reduce the number of initiated cells while the function of promoters is to facilitate cell proliferation of intiated cells.

APPENDIX 1: DERIVATION OF $\mu(t)$ AND $V(t)$ FOR THE EXTENDED TWO-STAGE MODEL OF TAN AND GASTARDO

Let $\psi(t_k) = \psi(u,v,t_k)$ be the PGF (probability generating functon) of intermediate cells and tumors at $t = t_k$ given a large number of normal cells at $t = 0$, and let $\phi_j(\xi_j) = \phi_j(u,v,\xi_j)$ be the PGF of intermediate cells and tumors at $t = t_j$ given one intermediate cell at $t = t_{j-1}$. Then, if each cell after the second mutation develops into a single tumor instantaneously, it is shown in Tan and Gastardo (*10*) that

$$\psi(t_k) = \exp\{\sum_{j=1}^{k}\alpha_{1j}\int_0^{\xi_j}\chi_j(s)[g_j(\xi_j - s) - 1]ds\}, \quad \text{(a.1)}$$

where $\chi_j(s)$ is the expected number of normal cells at $t_{j-1} + s$ given a large number of normal cells at time $t = 0$, $g_k(x) = \phi_k(u,v,x)$ and $g_j(x) = \phi_j[g_{j+1}(\xi_{j+1}),v,x]$, $j = 1, 2, \ldots, k-1$.

By taking derivative with respect to v over $\psi(t_k)$ and putting $u = v = 1$, one has:

$$\mu(t_k) = \sum_{j=1}^{k}\alpha_{1j}\int_0^{\xi_j}\chi_j(s)\mu_{1j}(\xi_j - s)ds, \quad \text{(a.2)}$$

where

$$\mu_{1j}(t) = \left[\frac{\partial}{\partial u}g_j(t)\right]_{u=v=1}$$

Putting for $j = 1, 2, \ldots, k-1$,

$$f_{1j}(t) = \left[\frac{\partial}{\partial v}\phi_j(u,1,t)\right]_{u=v=1} = \exp(\epsilon_j t),$$

$$f_{2j}(t) = \left[\frac{\partial}{\partial v}\phi_j(1,v,t)\right]_{v=1} = \alpha_{2j}[\exp(\epsilon_j t) - 1]/\epsilon_j$$

and

$$W_j = \mu_{1j}(\xi_j), \text{ where } \epsilon_j = b_j - d_j, \text{ then}$$
$$\mu_{1j}(t) = f_{1j}(t)W_{j+1} + f_{2j}(t), \quad \text{(a.3)}$$

and

$$W_j = A_j W_{j+1} + B_j,$$

where

$$A_j = f_{1j}(\xi_j) = \exp(\epsilon_j\xi_j) \text{ and}$$
$$B_j = f_{2j}(\xi_j) = \alpha_{2j}[\exp(\epsilon_j\xi_j) - 1]/\epsilon_j.$$

From (a.3) it follows that for $j = 1, \ldots, k$,

$$W_j = \sum_{u=j}^{k} B_u\left\{\prod_{v=j}^{u-1} A_v\right\}, \; W_{k+1} = 0$$

and

$$\mu_{1j}(t) = f_{1j}(t)W_{j+1} + f_{2j}(t)$$
$$= f_{1j}(t)\sum_{u=j+1}^{k} B_u\left\{\prod_{v=j+1}^{u-1} A_v\right\} + f_{2j}(t), \quad \text{(a.4)}$$

for $j = 1, \ldots, k$, where $\prod_1^0$ is defined as 1 and $\sum_{u=k+1}^{k}$ is defined as 0.

By taking the second derivative with respect to v over $\psi(t_k)$ and putting $u = v = 1$, one has:

$$\mu_2(t) = \sum_{j=1}^{k}\alpha_{1j}\int_0^{\xi_j}\chi_j(s)\mu_{2j}(s)ds + \mu_1(t), \quad \text{(a.5)}$$

where

$$\mu_{2j}(t) = \left[\frac{\partial^2}{\partial v^2} g_j(t)\right]_{u=v=1}$$

Put, for $j = 1, 2, \ldots, k$:

$$h_{1j}(t) = (2b_j/\epsilon_j)\exp(\epsilon_j t)[\exp(\epsilon_j t) - 1],$$
$$h_{2j}(t) = (2\alpha_{2j}/\epsilon_j^2)\exp(\epsilon_j t)[-\epsilon_j(b_j + d_j)t - 2b_j + 2b_j\exp(\epsilon_j t)], \quad \text{(a.6)}$$
$$h_{3j}(t) = (\alpha_{2j}^2/\epsilon_j^3)\{\epsilon_j\exp(\epsilon_j t) - \epsilon_j - 2\epsilon_j(b_j + d_j)t\exp(\epsilon_j t) + 2[d_j + b_j]/[\exp(\epsilon_j t) - 1]\},$$
$$H_j = h_{1j}(\xi_j),\ G_j = h_{2j}(\xi_j) \text{ and } F_j = h_{3j}(\xi_j).$$

Then, with

$$U_j = \mu_{2j}(\xi_j)$$
$$U_j = H_j W_{j+1}^2 + G_j W_{j+1} + A_j U_{j+1} + F_j, \quad \text{(a.7)}$$
$$j = 1,\ldots,k, \quad U_{k+1} = 0;$$
$$\mu_{2j}(t) = h_{1j}(t)W_{j+1}^2 + h_{2j}(t)W_{j+1} + h_{3j}(t) + f_{1j}(t)U_{j+1}, \quad \text{(a.8)}$$
$$j = 1, \ldots, k.$$

From (a.7), one has

$$U_j = \sum_{u=j}^{k-1}(H_u W_{u+1}^2 + G_u W_{u+1} + F_u)\prod_{v=j}^{u-1} A_v + F_k\left(\prod_{v=k}^{k-1} A_v\right),$$

$j = 1, \ldots, k$, $U_{k+1} =$ 0, where $\sum_{u=k}^{k-1}$ is defined as 0 and $\prod_{v=k}^{k-1}$ defined as 1.

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